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The molecular dynamics of *Trypanosoma brucei* UDP-galactose 4'-epimerase a drug target for African sleeping sickness

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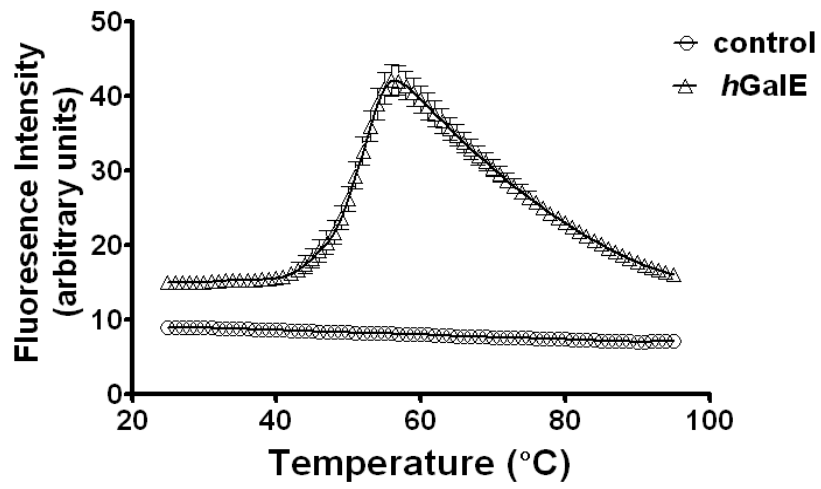
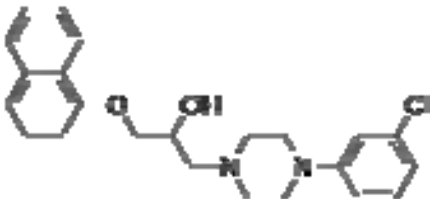
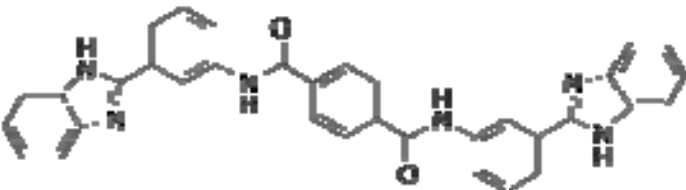
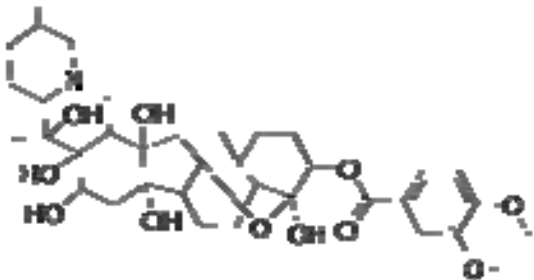
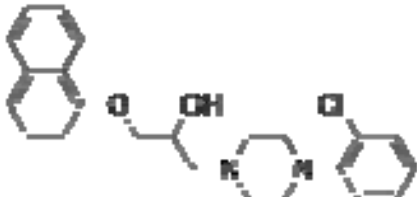
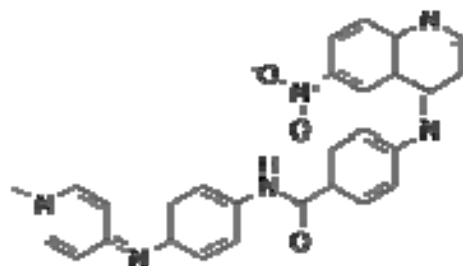


Figure S1. Thermal scanning fluorimetry of *HsGalE*. 5 μ M *HsGalE* in 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO, 5 \times Sypro orange showed a clear melting curve resulting in a T_m of 51.5 ± 0.3 °C.

Table S1. *Tb*GalE Agonists

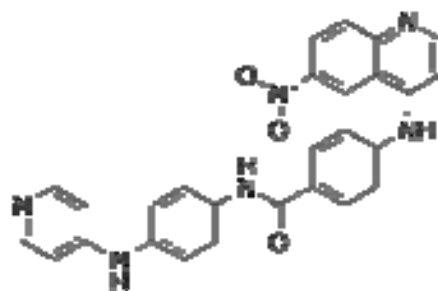
NSC ID	Structure	% inhib. @ 100 mM
91395		-167
61610		-169
7524		-191
91396		-194

260594



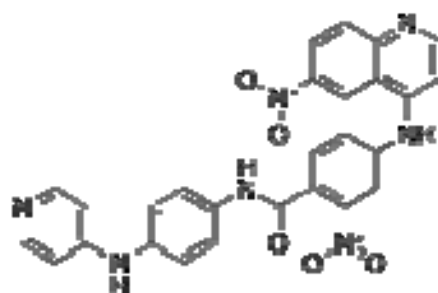
-223

146771



-242

202386



-283

Specific methods can be found in Durrant et al. (2010) *J Med Chem* 53, 5025-5032.

Table S2. Percentage activity of 20 nM *HsGalE* in the presence of different DTP compounds

DTP Compound	% Activity
No compound	100 ± 17
91395	102 ± 32
61610	89 ± 27
7524	112 ± 3
91396	124 ± 30
260594 ^a	30 ± 190
146771 ^a	104 ± 57
202386 ^a	41 ± 59

The reactions contained 100 µM DTP compound, 100 µM UDP-Galactose, 10 mM NAD⁺, 1.2 µM HsUGDH, 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO. Data are reported as the mean ± SD determined from three separate experiments. No compound resulted in a statistically significant (Student's t-test) change in activity.

^aCompounds 260594, 146771 and 202386 gave large errors due the formation of a coloured precipitate, which prevented accurate determination of activity.

Table S3. Melting temperatures of *HsGalE* in the presence of different DTP compounds

DTP Compound	T _m (°C)	ΔT _m (K)
No compound	51.5 ± 0.3	N/A
91395	51.3 ± 0.3	– 0.2 ± 0.6
61610	51.4 ± 0.1	– 0.1 ± 0.4
7524	51.3 ± 0.4	– 0.2 ± 0.7
91396	51.3 ± 0.3	– 0.2 ± 0.6
260594 ^a	N/D	N/D
146771 ^a	N/D	N/D
202386 ^a	N/D	N/D

The reactions contained 5 μM *HsGalE*, 100 μM DTP compound, 10 mM HEPES, pH 8.8, 1% (v/v) DMSO, 5× Sypro orange. The change of melting temperature, ΔT_m, due to ligand binding was calculated according to:

$$\Delta T_m = (T_m \text{ of protein without compound}) - (T_m \text{ of protein with compound})$$

Data are reported as mean ± SD determined from three experiments. If a compound bound to the enzyme, it would be expected to stabilize the protein's structure resulting in an increase in T_m. However, none of the compounds tested here resulted in a statistically significant (Student's t-test) change in T_m.

^aCompounds 260594, 146771 and 202386 formed a colored precipitate, preventing determination of the melting temperature.